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phillip gambel
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Wright, G.L.,Jr.; Haley, C.; Beckett, M.L.; Schellhammer, P.F.
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Original Articles

Expression of Prostate-Specific Membrane Antigen in Normal, Benign, and Malignant Prostate Tissues

George L. Wright, Jr, PhD,*†‡§ Cara Haley, BS,*‡§ Mary Lou Beckett, MS,*‡§ and Paul F. Schellhammer, MD*†‡§

Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein recognized by the murine monoclonal antibody (MAb) 7E11-C5.3 both in its native (CYT-351) and immunoconjugate form (CYT-356). Previous studies have shown that tissue expression of PSMA is highly restricted to prostate tissues. In this study, a definitive immunohistochemistry evaluation was performed to assess PSMA expression in prostate tissues. A stain index was established by multiplying the percentage of stained cells by the intensity of the stained cells to provide a quantitative measurement of PSMA expression in the various tissue types. The cellular location of PSMA, its correlation with clinical status, and its comparison with the expression of prostate-specific antigen (PSA) were evaluated. Prostate-specific membrane antigen was found to be highly expressed in most of the normal intraepithelial neoplasia, and the primary and metastatic prostate tumor specimens evaluated. In contrast to PSA, PSMA expression was often heterogeneous with variable staining patterns, ranging from a low-level diffuse cytoplasmic staining in normal prostate epithelium to very intense cytoplasmic and focal membrane staining in high-grade primary carcinomas and metastatic tissues. The predominant cytoplasmic staining was expected because the antigenic epitope of the PSMA transmembrane glycoprotein recognized by MAb 7E11-C5.3 is located in the cytoplasmic domain. Benign prostate tumors, ie, hypertrophy, showed the lowest expression of PSMA with a stain index of 52, compared with stain indexes of 146 and 258 for normal prostate and bone metastatic tissues, respectively. The reason for the apparent down-regulation of PSMA in benign prostate tissue is unknown but may be related to a splicing variant or post-translational modification of PSMA. Expression of PSMA was observed to increase with increasing pathologic grade, but not with clinical stage. Although PSMA was overexpressed in poorly differentiated and metastatic prostate tumors, expression in the primary tumor did not correlate with nodal status, extracapsular penetration, or seminal vesicle invasion. These results suggest that PSMA is not a useful biomarker of disease progression; however, high expression does appear to be associated with the more aggressive prostate carcinoma phenotype. The restricted specificity, differential prostate tissue expression, and overexpression of PSMA in metastatic tissues support the continued study of this unique prostate tumor-associated biomarker for developing new strategies for diagnosis and therapy of prostate cancer. *(Urol Oncol 1995;1:18–28)*

Prostate cancer is the most common (noncutaneous) cancer diagnosed in the American male and is steadily increasing, not only as a result of an increasing population of older men, but also because of greater awareness of the disease and earlier diagnosis using tumor markers such as prostate-specific antigen (PSA). It is projected that 200,000 men were diagnosed with prostate cancer in 1994,[1] representing a 34% increase in the number of prostate cancer cases (165,000) diagnosed in 1993. If the 1994 estimate is accurate, prostate cancer will become the most commonly diagnosed cancer, exceeding breast cancer (183,000) by 27,000 cases. More than 38,000 men are expected to die of prostate cancer in 1994, making deaths from prostate cancer second only to lung cancer deaths. Patients diagnosed with localized disease have far better survival rates than patients diagnosed with metastatic disease. Early detection of localized prostate cancer and improved treatment of metastatic disease are important strategies to reduce prostate cancer deaths.

Although serum PSA measurements have had a major impact on the diagnosis and management of prostate cancer,[2,3] PSA is far from being the ideal cancer marker. Twenty-five percent of patients with benign prostatic hypertrophy (BPH) present with elevated levels of PSA, approximately 30% of prostate cancer patients present with normal PSA values, and PSA expression is unable to differentiate biologically active from inactive cancers. These statistics suggest that other clinical markers are needed to improve early diagnosis, to identify aggressive tumors, and to develop new therapeutic strategies. A new prostate marker, prostate-specific membrane antigen (PSMA), may meet one or more of these objectives. Prostate-specific membrane antigen appears to be a transmembrane glycoprotein with a major Mr 100,000 component[4–6] recognized by monoclonal antibody (MAb) 7E11-C5.3.[7] Recently, the cDNA encoding PSMA was cloned, and the deduced amino acid sequence revealed a novel polypeptide

From the Departments of *Microbiology and Immunology and †Urology, and ‡The Virginia Prostate Center, Eastern Virginia Medical School, and §Sentara Cancer Institute, Norfolk, Virginia.

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Address correspondence to George L. Wright, Jr, PhD, Department of Microbiology and Immunology, Eastern Virginia Medical School, 700 Olney Road, Norfolk, VA 23501.

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655 Avenue of the Americas, New York, NY 10010

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structure.[4] Clinical trials using MAb 7E11-C5.3 conjugated to either ^{111}In or ^{90}Y are in progress for diagnostic imaging and therapy, respectively.[6–11] Previous reports[5,7–9] have shown that PSMA expression is highly restricted to prostate tissues and that the expression in normal prostate tissues appeared to be less than that in malignant prostate tissues. These studies, performed primarily to determine the specificity of PSMA expression, evaluated a small number of tissue specimens, and no relation of PSMA expression to clinical status was presented. In this report, we present a definitive description of the differential expression of PSMA in normal prostate, BPH, prostate intraepithelial neoplasia (PIN), and primary and metastatic prostate carcinoma (CaP) tissues, and the relation of PSMA expression to tumor grade and extraprostatic disease.

Materials and Methods

Tissues

Formalin-fixed, paraffin-embedded blocks of transurethral resected specimens of BPH, prostatectomy specimens of prostate carcinoma, and lymph node and bone metastatic tissues were obtained from the Virginia Prostate Center Tissue Bank. Normal prostate tissue was obtained from males aged 16 to 45 years with no evidence of prostate disease. These tissues were obtained by autopsy, usually within 12 hours after death, from the Cooperative Human Tissue Network, University of Alabama at Birmingham; and from the Norfolk Medical Examiner's Office. All tissue specimens for paraffin embedding were fixed in the same neutral buffered 10% formalin. Samples of normal, BPH, and prostate carcinoma tissues obtained directly from surgery or autopsy also were embedded in OCT compound in cryomolds and snap-frozen in isopentane over liquid nitrogen.

Monoclonal Antibodies

Affinity-purified MAb 7E11-C5.3 (referred to as native antibody or CYT-351) and an affinity-purified conjugated form of MAb 7E11-C5.3 (designated CYT-356[12]) were provided by CYTOGEN Corp. (Princeton, NJ). Monoclonal antibody EVMS-PSA-5 was produced in mice against purified PSA from pooled normal seminal plasma and affinity purified from ascites, following our published protocols.[13–15]

Immunoperoxidase Staining

The expression of PSMA and PSA in tissues was detected by the avidin-biotin peroxidase assay using the ABC Elite Vectastain kit (Vector Laboratories, Burlingame, CA), as described previously.[13–15] Briefly, 4-μm paraffin sections were cut, deparaffinized, and rehydrated through xylene and a graded series of alcohols. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol for 30 minutes. Frozen sections were cut at 6 μm, briefly dipped in cold acetone, and stored at −20°C until used, or air dried for 30 minutes before proceeding with the staining reaction. Endogenous peroxidase activity was blocked with 3.0% H_2O_2 in water for 5 minutes. From this point on, both frozen and paraffin-embedded sections were treated in the same manner. Nonspecific binding was blocked by incubation with 10% normal horse serum for 10 minutes, followed by a 30-minute incubation with the primary antibody, either MAb 7E11-C5.3 or PSA-5, followed by a 10-minute incubation with the biotinylated secondary antibody, and then the ABC complex. The optimal concentrations for the antibodies (20 μg/mL for MAb 7E11-C5.3 and 2 μg/mL for PSA-5) were predetermined by titrating the MAbs on normal, BPH, and prostate carcinoma tissues. These concentrations from the same antibody lot were used for evaluating all the specimens in this study. After development with the chromogen substrate 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO), the sections were counterstained with Mayer's hematoxylin and mounted in aqua mount (Learner Laboratories, Pittsburgh, PA). Antigen expression was scored by calculating the percentage of cells positive in a number of 20-mm objective views sufficient to cover all areas of the tissue section. Staining intensity was also recorded using a scale of 1 (low), 2 (moderate), and 3 (high). The stained tissue sections were scored independently by two investigators, with the two scores having a difference of less than 10%. A stain index was calculated by multiplying the mean percentage PSMA-positive cells by the mean staining intensity.

Results

Expression of PSMA and PSA in Normal, Benign, and Malignant Prostate Tissues

The binding of MAb 7E11-C5.3 was compared with the binding of a MAb to PSA using the immunoperoxidase assay on frozen and formalin-fixed, paraffin-embedded tissue sections of prostate specimens consisting of normal prostate, BPH, and malignant primary and metastatic prostate carcinomas. By optimizing the staining conditions, both frozen (data not shown) and paraffinized tissue specimens from the same patient gave identical staining patterns for MAb PSA-5 and the native and immunoconjugate forms of MAb 7E11-C5.3. The use of paraffin-embedded tissues enabled us to conduct a large retrospective study to evaluate MAb 7E11-C5.3 reactivity on normal, benign, PIN, and malignant prostate tissues. The epithelial cells of all four prostate tissue types were found to express both the PSMA and PSA antigens (Table 1; Figures 1–4). As expected, nearly all the prostate specimens expressed PSA, with the exception of the bone marrow metastatic specimens, of which only 57% stained positive for PSA. The mean percentage of epithelial cells expressing PSA ranged from 80–98% for all prostate tissue types, with the exception of the bone metastatic specimens (48%). Ninety-one to 100% of the prostate tissues expressed PSMA, with the exception of the BPH specimens, in which PSMA expression was positive in only 22 of 27 (81%). The mean percentage of epithelial cells expressing PSMA in the PIN specimens was 59%, and in the primary prostate carcinomas was 53%. The highest expression was found in the metastatic tumors: 72% for lymph nodes and 92% for bone metastasis. Of special interest was the observation that the highest percentage

TABLE 1. COMPARISON OF PROSTATE-SPECIFIC MEMBRANE ANTIGEN WITH PROSTATE-SPECIFIC ANTIGEN EXPRESSION IN FORMALIN-FIXED, PARAFFIN-EMBEDDED PROSTATE TISSUES

	PSMA		PSA	
Tissue	No. positive/ no. tested	Mean % positive cells	No. positive/ no. tested	Mean % positive cells
Normal	12/12 (100%)	77	12/12 (100%)	98
BPH	22/27 (81%)	29	27/27 (100%)	95
PIN	21/21 (100%)	59	21/21 (100%)	98
CaP	157/165 (95%)	53	161/165 (98%)	81
LN mets	72/79 (91%)	72	74/79 (94%)	81
Bone mets	7/7 (100%)	92	4/7 (57%)	48

PSMA = prostate-specific membrane antigen; PSA = prostate-specific antigen; BPH = benign prostatic hyperplasia; PIN = prostatic intraepithelial neoplasia; CaP = primary prostatic carcinoma; LN = lymph node; Mets = metastasis.

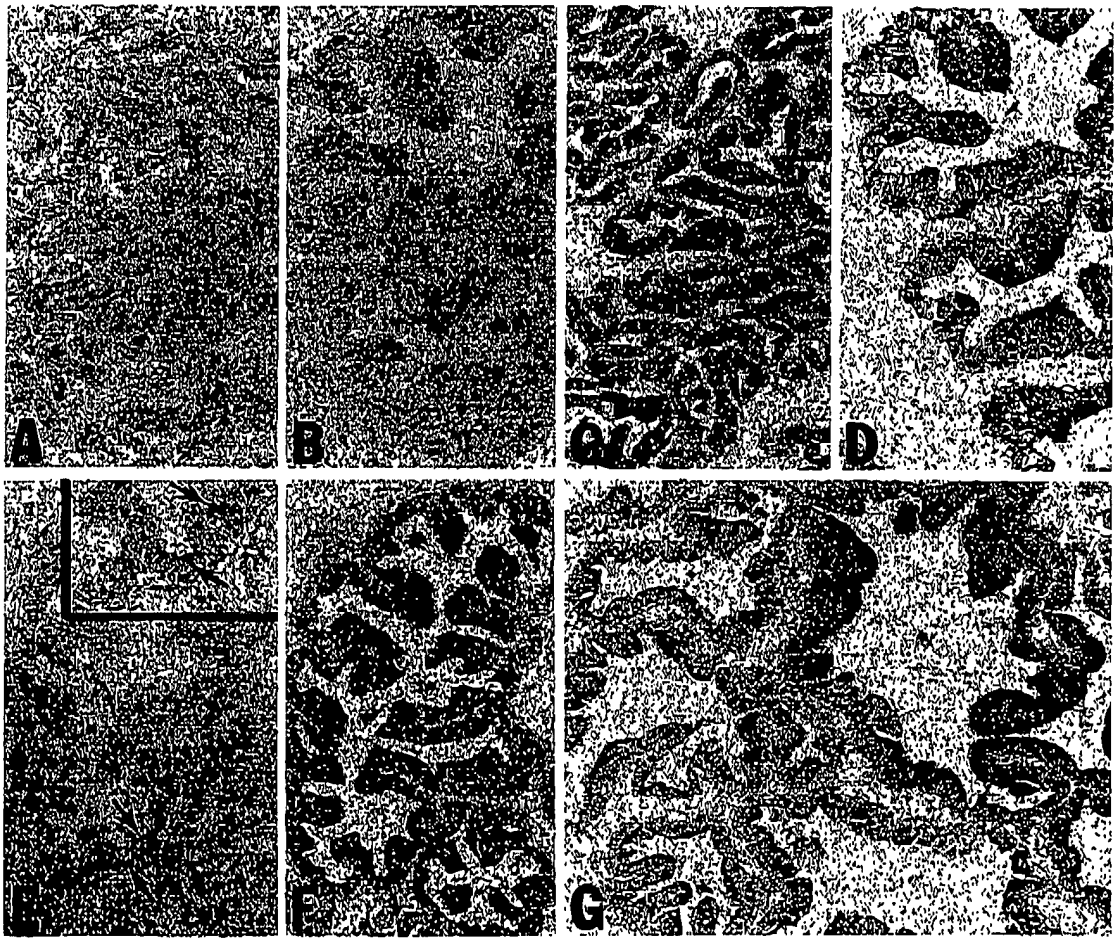

FIGURE 1. Immunoperoxidase staining of normal, benign (BPH), and PIN tissues with monoclonal antibodies to either PSMA or PSA. A–D) Normal prostatic tissues. A) and B) stained for PSMA; C) and D) stained for PSA. Note diffuse cytoplasmic and low staining intensity for PSMA. E) and F) BPH tissue stained for PSMA (E) and PSA (F). Note scattered focal membrane staining for PSMA (*arrows*). G) PIN stained for PSMA. Original magnification: ×100 (A,C); ×200 (B,D,E,F,G); ×400 (*E Inset*).

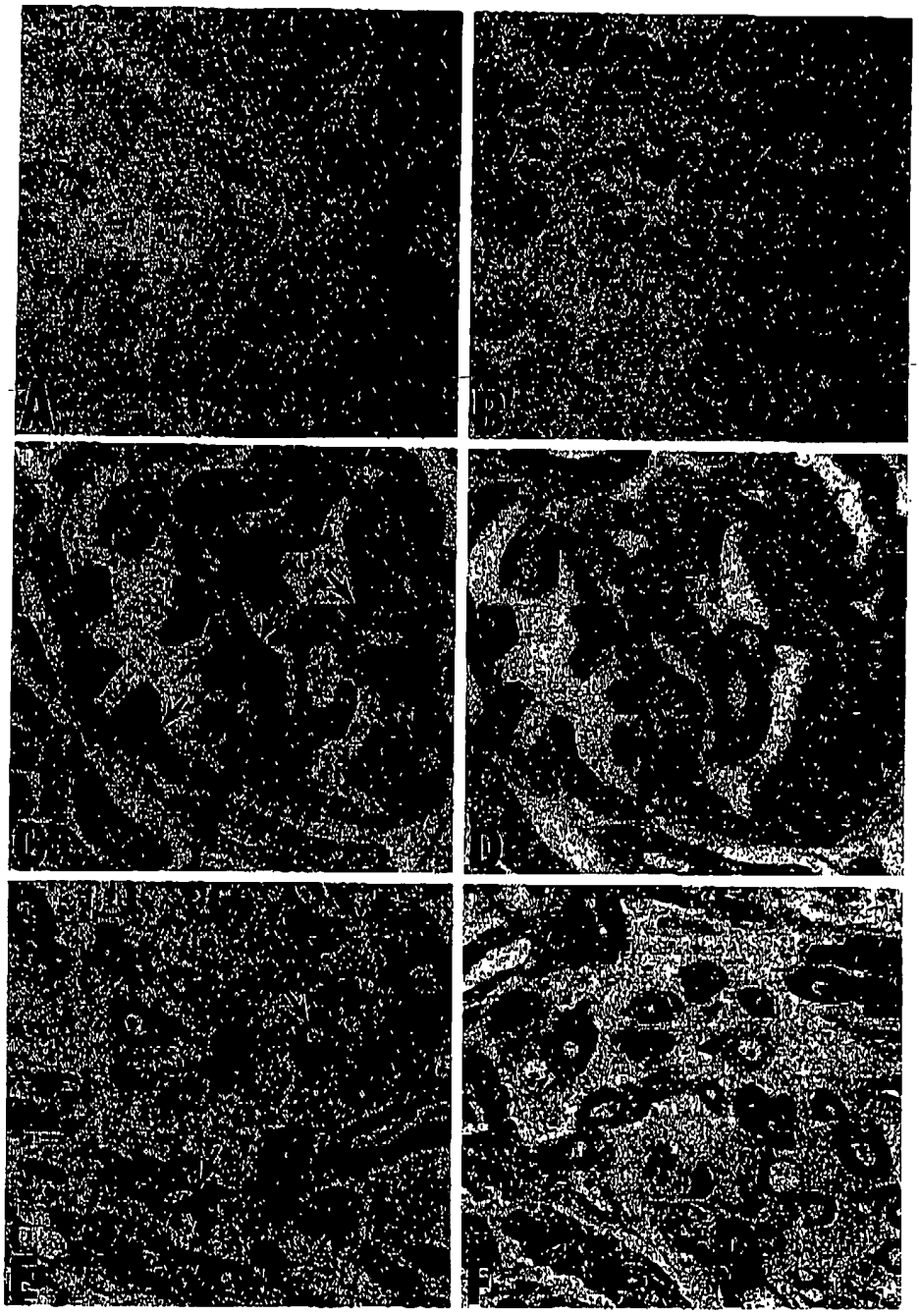

FIGURE 2. Prostate carcinoma tissues stained with monoclonal antibody to either PSMA or PSA. A) and B) Well-differentiated carcinoma; C) and D) moderately differentiated carcinoma; E) and F) poorly differentiated carcinoma. A), C), and E) stained for PSMA; B), D), and F) stained for PSA. Note the minimal and low-intensity cytoplasmic staining for PSMA in the well-differentiated carcinoma, with increase in the number of cells and staining intensity with increasing tumor grade, and intense focal and luminal membrane staining (*arrows*). Original magnification: ×200 (A–F).

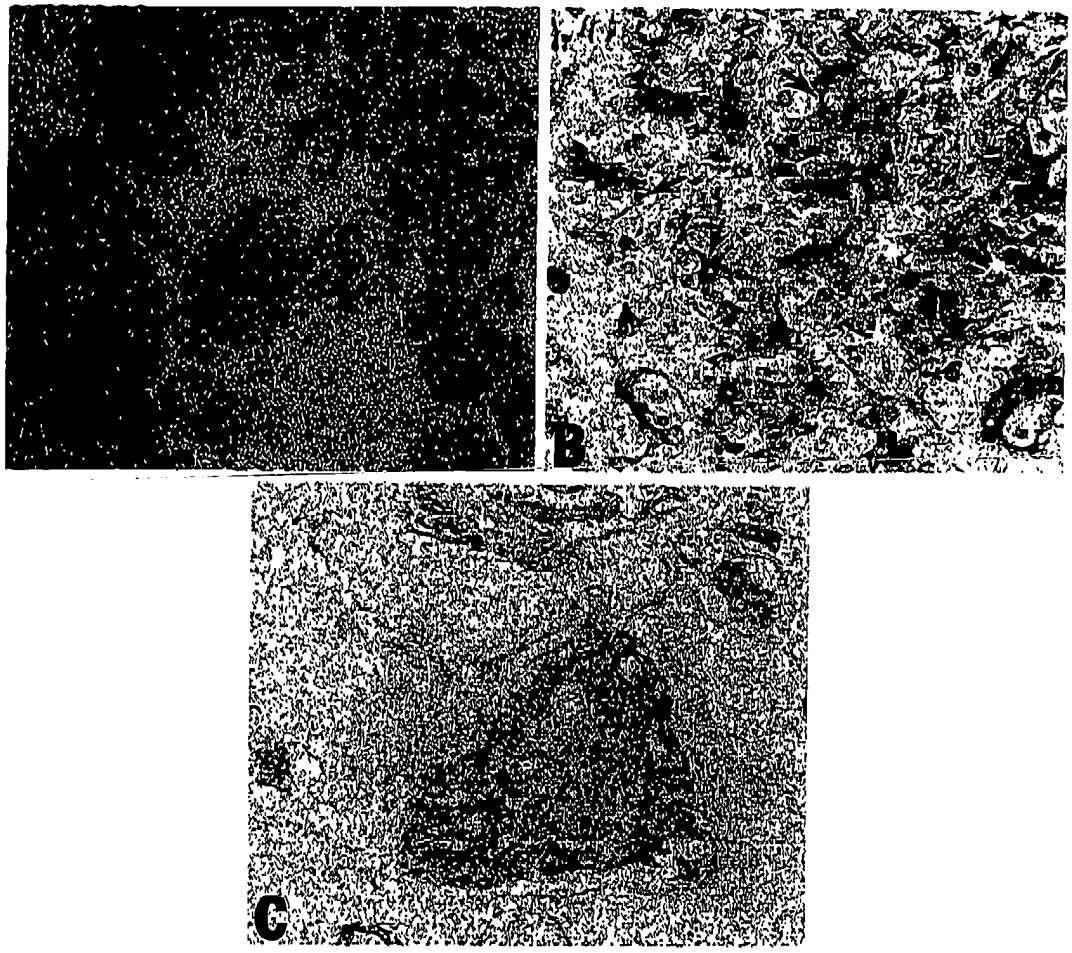

FIGURE 3. Metastatic prostate tissues stained for PSMA. A) and B) Lymph node metastatic tissue. C) Bone metastatic tissue. B) is a higher magnification showing intense focal and luminal membrane staining (*arrows*) associated with minimal cytoplasmic staining. Original magnification: ×100 (A,C); ×400 (B).

(77%) of epithelial cells expressing PSMA was in the normal prostate specimens, and the lowest percentage of positive cells (29%) occurred in the BPH specimens.

Expression and Cellular Localization of PSMA in Prostate Tissues

We evaluated PSMA expression in prostate tissues further by taking into consideration both the percentage and the staining intensity of prostate epithelial cells. A stain index was calculated by multiplying the mean percentage of cells expressing PSMA by the staining intensity (1 = low; 2 = moderate; 3 = high intensity). With this approach, BPH specimens clearly had the lowest stain index (SI = 52), ie, the lowest number of positive epithelial cells and the lowest staining intensity (Table 2, Figure 1E). Both the number of stained cells and the staining intensity increased in the PIN (Figure 1G) and the malignant prostate specimens (Figure 2), with the highest index (SI = 258) determined for the bone metastatic specimens. Although the normal prostate tissues had a high stain index (SI = 146), the staining pattern was quite different from that of the other tissue specimens. In these specimens, PSMA expression was predominantly diffuse and cytoplasmic with low to moderate staining intensity (Table 2; Figure 1B), with an occasional duct or luminal cell showing luminal membrane staining. Of all prostate tissues examined, BPH demonstrated the greatest antigenic heterogeneity. In contrast to normal prostate, few luminal cells expressed PSMA and the expression was often focal, with some apical membrane staining and minimal cytoplasmic staining (Figure 1E). The PIN lesions had a stain index (SI = 130) similar to normal prostate but showed a more intense, diffuse cytoplasmic and luminal membrane staining (Figure 1G). The stain index (SI = 133) for PSMA expression in the 165 primary prostate carcinomas evaluated, including all histopathologic grades, was similar to the index for normal and PIN tissues (Table 2). Cellular localization of PSMA in these tissues ranged from diffuse cytoplasmic staining in the well-differentiated speci-

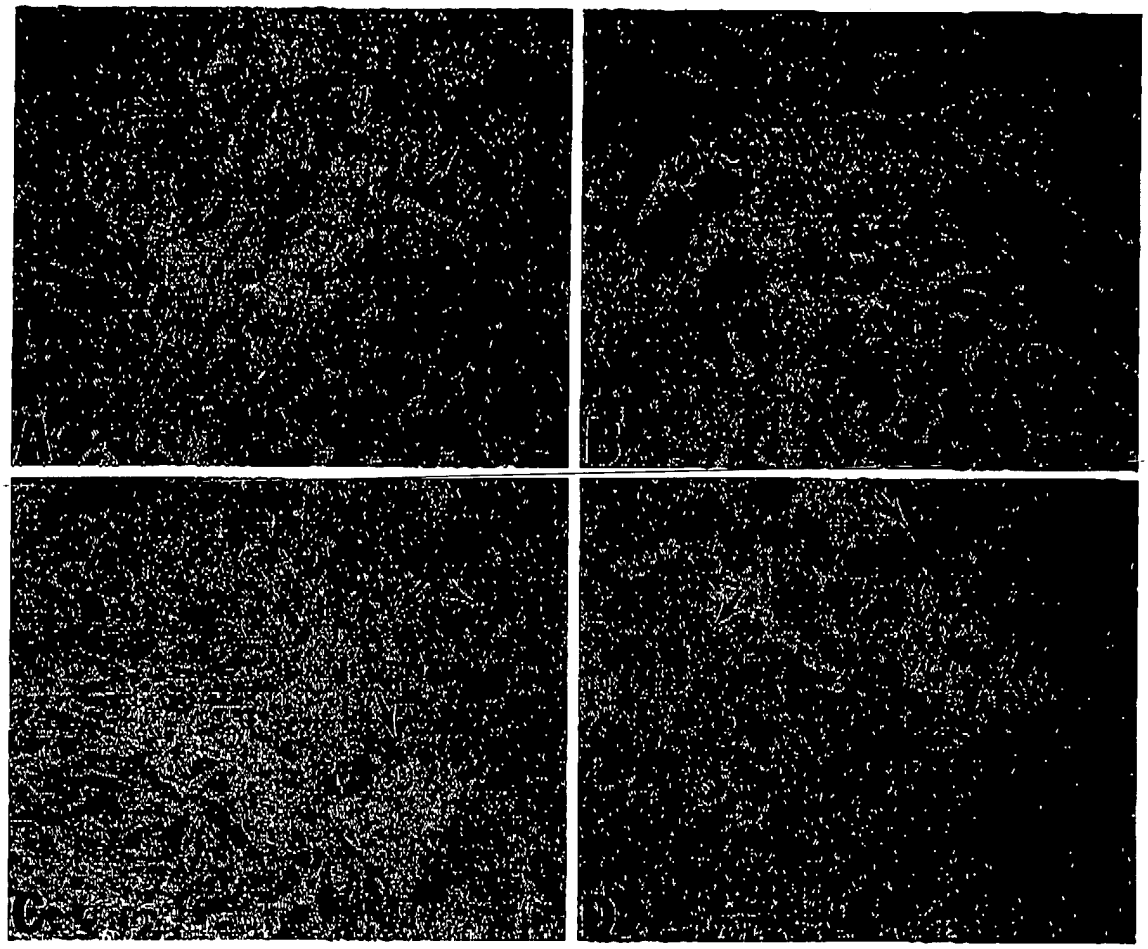

FIGURE 4. Immunoperoxidase staining of three representative prostate carcinomas to illustrate the differential PSMA expression in various tissue types in the same specimen. A) and B) are serial sections from one patient; C) and D) are sections from two different patients, respectively. A), C), and D) were stained for PSMA and B) was stained for PSA. Note minimal to no staining in the normal/benign and well-differentiated (*arrows*) areas, compared with the intense focal and luminal membrane staining with some cytoplasmic staining (particularly in section D) in the poorly differentiated carcinoma areas. Note that all tissue areas stained for PSA (B). Original magnification: ×100 (A–D).

TABLE 2. DIFFERENTIAL EXPRESSION OF PROSTATE-SPECIFIC MEMBRANE ANTIGEN IN FORMALIN-FIXED, PARAFFIN-EMBEDDED PROSTATE TISSUES

Tissue	No. positive/ no. tested	% positive cells[a]	Intensity[a]	Stain index[b]	PSMA localization[c]			
					DC	FC	F/M	LE
Normal	12/12 (100%)	77 ± 32	1.9 ± 0.33	146	+ +			+
BPH	22/27 (81%)	29 ± 29	1.8 ± 0.90	52	+		+ +	+
PIN	21/21 (100%)	59 ± 21	2.2 ± 0.37	130	+		+ +	
CaP	157/165 (95%)	53 ± 32	2.5 ± 0.66	133	+ +		+ +	+ + +
LN mets	60/64 (94%)	72 ± 36	2.7 ± 0.92	194	+	+ +	+ + +	+ + +
Bone mets	7/7 (100%)	92 ± 10	2.8 ± 0.40	258	+	+ +	+ + +	+ + +

DC = diffuse cytoplasmic; FC = focal areas within the cytoplasm; F/M = membrane or focal membrane; LE = edge of luminal cells; other abbreviations as in Table 1.

[a]Mean ± SD.

[b]Stain index calculated by multiplying the mean percentage of cells staining by the staining intensity.

[c]+ = low staining; ++ = moderate staining; +++ = high staining.

TABLE 3. PROSTATE-SPECIFIC MEMBRANE ANTIGEN EXPRESSION VERSUS GRADE

Gleason sum	No. positive/ no. tested	Mean % cells positive	Mean stain intensity	Stain index
2–4 (WD)	22/26 (85%)	48	2.3	110
5–7 (MD)	92/100 (92%)	50	2.5	125
8–10 (PD)	37/39 (95%)	62	2.6	161

WD = well-differentiated; MD = moderately differentiated; PD = poorly differentiated.

mens (Figure 2A) to intense focal and luminal membrane staining in the moderate (Figure 2C) to high-grade carcinomas (Figure 2E). Perinuclear staining was sometimes observed in a few malignant epithelial cells in some of the high-grade specimens. Staining heterogeneity was evident for all tumor grades, with the highest degree observed in the low to moderate grades. The highest PSMA expression, in terms of number of cells staining and intensity of staining, was observed in the metastatic tissues (Table 2; Figure 3). As shown in Figure 3, the predominant cellular expression of PSMA in the lymph node and bone metastatic specimens was intense apical membrane staining, often with minimal cytoplasmic staining (Figure 3). Less common was the finding of occasional perinuclear staining and intensely stained focal areas within the cytoplasm (not shown). The PSMA staining pattern observed in the metastatic tissues was similar to that for the moderate to high-grade prostate carcinoma specimens (Figure 2C and E); however, there was considerable less staining heterogeneity in the metastatic tissues.

PSMA Expression by Tumor Grade

Table 3 shows the expression of PSMA in the different pathologic grades. The Gleason scores of all 165 primary prostate carcinomas were placed into three groups representing the three general pathologic differentiation grades. Although not remarkable, a slight but positive correlation of PSMA expression with tumor grade was observed. The differential expres-

TABLE 4. COMPARISON OF PROSTATE-SPECIFIC MEMBRANE ANTIGEN EXPRESSION IN LOW-GRADE AND HIGH-GRADE TUMOR AREAS ON THE SAME PROSTATE CARCINOMA TISSUE SPECIMEN

Tissue specimen	Low grade			High grade		
	%Cell[a]	In[b]	Index[c]	%Cell[a]	In[b]	Index[c]
CA153	20	1	20	95	3	285
CA1061	20	1	20	98	2.5	245
CA8750	10	1	10	70	3	210
CA5262	20	1	20	75	3	225
CA1124	70	3	210	90	3	270
CA1197	35	1	35	100	3	300
CA1789	50	1	50	50	2	100
CA8331	20	1	20	98	3	294
CA1022	5	1	5	90	2.5	225
CA2048	55	3	165	100	2	200
CA7506	70	2	140	95	3	285
CA4319	5	1	5	90	2	180
CA4475	35	2	70	90	3	270
CA3651	10	2	20	80	3	240
CA3984	20	1	20	60	3	180
CA8725	50	3	150	90	3	270
CA1471	85	2	170	100	3	300
CA6166	10	1	10	95	3	285
CA0600	40	2	80	70	3	210
CA5850	100	1	100	100	3	300
CA1977	65	3	195	80	3	240
CA1709	10	1	10	99	2.5	248

[a]%Cell = percentage of tumor cells staining.
[b]In = staining intensity (1 = low; 2 = moderate; 3 = strong).
[c]Staining index, calculated by multiplying the mean percentage of positive cells by staining intensity.

sion of PSMA in low- and high-grade carcinomas was more accurately accessed when separate indexes were calculated for the l w- and high-grade areas contained in the same tumor specimen. Table 4 shows the results of PSMA expression in 22 randomly selected high-grade carcinomas containing focal areas of low-grade carcinoma. In all 22 cases, the stain index for the high-grade areas (mean SI = 244) was higher than that for the low-grade areas (mean SI = 59). Figure 4 shows the differential staining patterns in three carcinoma specimens containing both high- and low-grade tumor areas. Strong staining was observed in the poorly differentiated areas, often with minimal to no staining of the low-grade areas and the normal/benign areas. No correlation was found between PSMA expression and clinical or pathologic stage (data not shown).

PSMA Expression in Lymph Node Metastases

The high PSMA expression in metastatic lymph nodes (94% of 64 positive nodes, SI = 194; Table 2) suggested that PSMA expression in primary carcinomas may represent a biomarker of metastatic progression. Although the percentage of tumor cells expressing PSMA often was increased in the metastatic lymph node (Table 5), the patient's primary carcinoma did not reflect this PSMA activity; therefore, PSMA staining in the primary tumor was not predictive of nodal status (Table 6). Similarly, PSMA expression did not correlate with positive margins, extracapsular penetration, or seminal vesicle invasion (data not shown). Correspondingly, PSA expression also did not correlate with these pathologic parameters (data not shown).

Discussion

Prostate-specific membrane antigen is expressed as a prominent Mr 120,000 transmembrane glycoprotein in prostate tissue extracts and seminal plasma.[4–6] It is detected using the mouse MAb 7E11-C5.3, produced against a membrane extract of LNCaP cells.[7,16,17] Immunostaining of normal and malignant tissues demonstrated that PSMA expression is highly restricted to prostate tissues[7,12] (also Wright GL Jr, Haley C,

TABLE 5. EXPRESSION OF PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND PROSTATE-SPECIFIC ANTIGEN IN THE PATIENT'S PRIMARY PROSTATE CARCINOMA AND LYMPH NODE METASTASIS

	Primary carcinoma			Metastatic lymph nodes	
		Percent cells		Percent cells	
Patient	Gleason[a] sum	PSMA	PSA	PSMA	PSA
CA2577	4	0	90	10	25
CA1290	4	5	40	20	70
CA5624	5	70	100	5	5
CA4306	5	10	95	75	100
CA2149	6	5	90	0	95
CA2071	7	15	100	45	95
CA9970	7	40	95	95	95
CA1842	7	40	10	75	25
CA5972	7	85	98	98	93
CA6918	7	20	100	100	90
CA4495	7	95	100	100	100
CA5371	7	25	10	5	30
CA8170	7	45	100	90	50
CA1197	7	00	95	100	100
CA1064	7	60	85	65	100
CA6136	8	65	100	40	100
CA1007	8	90	100	80	95
CA1435	8	70	100	100	90
CA1640	8	10	40	95	50
CA1602	8	55	90	95	80
CA4475	8	75	100	100	100
CA1360	8	5	100	5	75
CA1551	8	85	90	75	95
CA5750	8	80	100	100	90
CA8292	8	75	90	25	25
CA3411	9	30	35	25	80
CA3984	9	70	100	90	100

PSMA = prostate-specific membrane antigen; PSA = prostatic-specific antigen.
[a]Gleason sum: 1–4 = well-differentiated; 5–7 = moderately differentiated; 8–10 = poorly differentiated.

TABLE 6. PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND PROSTATE-SPECIFIC ANTIGEN EXPRESSION IN PRIMARY CARCINOMA VERSUS NODE STATUS

	PSMA			PSA		
Node status	No. tested	No. positive	Mean % positive cells[a]	No. tested	No. positive	Mean % positive cells[a]
Positive	21	21	54 ± 32.1	21	21	86 ± 26.2
Negative	78	75	49 ± 32.0	78	77	76 ± 27.0

Abbreviations as in Table 5.
[a]Mean ± SD.

Beckett ML, unpublished results). Early immunohistochemistry studies showed that MAb 7E11-C5.3 bound the type-2 muscle fibers of normal skeletal muscle; however, a ^{111}In-labeled immunoconjugate (CYT-356) of MAb 7E11-C5.3 failed to localize to skeletal muscle.[12] Recent studies in our laboratory (Troyer JK, Feng Q, Beckett ML, Wright GL Jr, unpublished results) and at Sloan-Kettering[18] have shown that neither the PSMA glycoprotein nor the PSMA mRNA could be detected in tissue extracts of normal skeletal muscle, suggesting that the observed immunostaining in skeletal muscle is entirely nonspecific. Further studies from these laboratories have shown mRNA[18] and PSMA (Troyer et al, unpublished results) in extracts of normal brain, salivary gland, and small intestine by blotting procedures, but not by immunohistochemistry of frozen or formalin-fixed tissue sections[7,12] (also Wright et al, unpublished results). These results suggest either that PSMA expression is below the detection limits of the immunohistochemistry assay or that post-translational modifications mask the PSMA epitope in these tissues. Previous immunohistochemistry studies focused on evaluating the specificity of MAb 7E11-C5.3 tissue reactivity. The present study provides a definitive descriptive immunohistochemistry examination of PSMA expression in normal, benign, and malignant prostate tissues.

Immunoreactivity for PSMA was detected in all types of prostate epithelium, confirming organ specificity rather than prostate carcinoma specificity of this biomarker. Expression of PSMA and PSA in all tissue specimens, with the exception of BPH and bone metastases, paralleled each other. Expression of PSMA in the majority of BPH specimens appeared to be both very heterogeneous and down-regulated. These immunostaining results correlate with the recent observation that PSMA mRNA levels are low to absent in BPH, even though they are high in both normal and malignant prostate tissues.[18] The reason for this phenomenon has yet to be determined. Recent evidence localizing the antigenic epitope recognized by MAb 7E11-C5.3 in the cytoplasmic domain of the PSMA glycoprotein (see Troyer et al, this issue)[19] may indicate that a splicing variant involving the N-terminal amino acid sequence could be responsible for the low PSMA expression in BPH tissues. New antibodies to different PSMA epitopes may assist in addressing this question. Further studies will be required to evaluate this or alternative hypotheses to explain the low PSMA expression in BPH tissues.

The pattern and localization of immunostaining were variable for all prostate tissues examined, with cytoplasmic immunoreactivity observed in all prostate epithelial cells. In contrast to a diffuse cytoplasmic staining, luminal membrane staining was found in PIN and primary and metastatic carcinoma tissues, with the most prominent membrane staining observed in poorly differentiated primary carcinomas and metastatic tissues. Based on the calculated stain indexes, PSMA was markedly overexpressed in the primary tissues with a high Gleason sum and in both metastatic lymph node and bone lesions. However, in contrast to primary tumors, the metastatic tissues demonstrated less staining heterogeneity. The reason for this apparent up-regulation with more uniform expression in the metastatic tissues is unknown. The effect of hormones on PSMA expression is currently being evaluated.

Although PSMA is an integral transmembrane protein, the cytoplasmic staining observed in prostate epithelium, especially in normal prostate tissues and well-differentiated tumors, could be explained by the location of the epitope in the cytoplasmic domain. Preliminary studies in our laboratory, using both light and electron immunomicroscopy, have demonstrated intracellular as well as membrane staining in cultured LNCaP cells.[20,21] Immunoelectron microscopy showed MAb 7E11-C5.3 localization at the internal region of the plasma membrane, confirming the mapping of the antigenic epitope to the intracellular domain. Besides binding at the internal plasma membrane, MAb 7E11-C5.3 also localized to certain cytoplasmic organelles. Further studies are in progress to determine whether the cellular localization of PSMA observed in LNCaP cells also occurs in prostate tissues. In any event, strong evidence is presented that PSMA is largely expressed intracellularly (ie, intracellular organelles) and at the cytoplasmic face of the plasma membrane of LNCaP cells and prostate tissues.

The observed cytoplasmic staining pattern and localization raise the question of how the ^{111}In-labeled 7E11-C5.3 immunoconjugate (^{111}In-CYT-356) is able to image prostate cancer in vivo.[8,9] Epitope-mapping experiments conducted in our laboratory have yet to demonstrate an epitope recognized by MAb 7E11-C5.3 in the extracellular domain of the PSMA glycoprotein (Troyer et al, unpublished results). As stated above, the only epitope recognized by MAb 7E11-C5.3 is located in the cytoplasmic domain. The sequence for this epitope is not found in the extracellular polypeptide region. It is quite possible, however, that the MAb binds to a similar but lower-affinity epitope expressed in the extracellular domain, thereby explaining successful imaging of the prostate cancer. Based on the amino acid sequence, there are numerous glycosylation sites available, suggesting that the extracellular peptide is heavily glycosylated. If this is true, then glycosylation may in fact mask the binding of MAb 7E11-C5.3 to these epitopes. Furthermore, carbohydrates are not part of the

epitope recognized by MAb 7E11-C5.3.Only the linear N-terminal peptide region is required for antibody binding. Based on these observations, the only mechanism for binding of the immunoconjugate to its antigenic target would be by binding to shed antigen in the intercellular spaces or passing through the plasma membrane to reach the epitope. The latter may be possible if the cells are undergoing apoptosis or necrosis. It is entirely possible that the intensely stained focal deposits in the cytoplasm of some malignant cells may represent apoptosis. This possibility is currently being explored. We know that PSMA is shed into prostatic fluid and is present in seminal plasma (Troyer et al, unpublished results), but we have not been able to confirm the initial observations[7] that PSMA is also shed in serum. Further studies will be required to elucidate fully how the ^{111}In-CYT-356 immunoconjugate images prostate carcinomas.

Expression of PSMA appeared to correlate with tumor grade. When separate stain indexes were calculated for a random cohort of high-grade tumors (SI = 244) containing focal areas of low-grade tumor (SI = 59), the marked overexpression of PSMA in the high-grade areas became more clearly evident. In contrast, 90–100% of all prostate epithelial cells in these tissues intensely expressed PSA. In this study, PSMA expression did not correlate with pathologic stage. However, the majority of tumors examined were stage C disease. Because of the marked overexpression observed in poorly differentiated and metastatic prostate tumors, it might be expected that PSMA expression would correlate with the more aggressive and advanced stage D2 tumors. Additional studies will be required to determine whether this is the case.

Based on our initial observations of the intense membrane expression in the high-grade areas of primary tumors plus the overexpression in the metastatic tumor specimens, we postulated that this pattern of PSMA expression in the primary carcinomas would predict metastasis or tumor progression. However, this was not the case. Evaluation of the nodal status of 99 prostate cancer patients failed to show any correlation with the expression of PSMA in their primary carcinoma; nor was PSMA expression in the primary tumor predictive of extracapsular penetration or seminal vesicle invasion. Because most of the prostate carcinoma specimens were from patients who had had a radical prostatectomy, no attempt could be made in this series to determine the effects of radiation and hormone deprivation therapy on PSMA expression. However, we have noted in preliminary studies that hormone ablation therapy either has no effect on or up-regulates PSMA expression (Grob RM, Haley C, Newhall K, Schellhammer PF, Wright GL Jr, unpublished results). The effect of hormone ablation therapy on PSMA expression will be the subject of a separate report.

This study demonstrates the differential expression of PSMA in normal, benign, and malignant prostate tissues. The unexpectedly low expression in BPH tissues, as compared with normal and malignant prostate tissues, deserves further evaluation to determine the mechanism for the low expression and to exploit this observation as a possible means to differentiate BPH from CaP. We found that PSMA was overexpressed in the poorly differentiated and metastatic tumors. Although PSMA expression in the primary tumor was not predictive of metastatic disease, high expression appears to be associated with the more aggressive prostate tumor, especially for hormone-refractory cancers. Because of the prominent intracellular location of the antigenic epitope, a mechanism to explain the clinical success of radiologic imaging with CYT-356 remains uncertain. Nevertheless, the restricted specificity, differential prostate tissue expression, and overexpression of PSMA in metastatic tissues support the continued study of this unique prostate tumor-associated biomarker for developing new strategies for the diagnosis and therapy of prostate cancer.

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